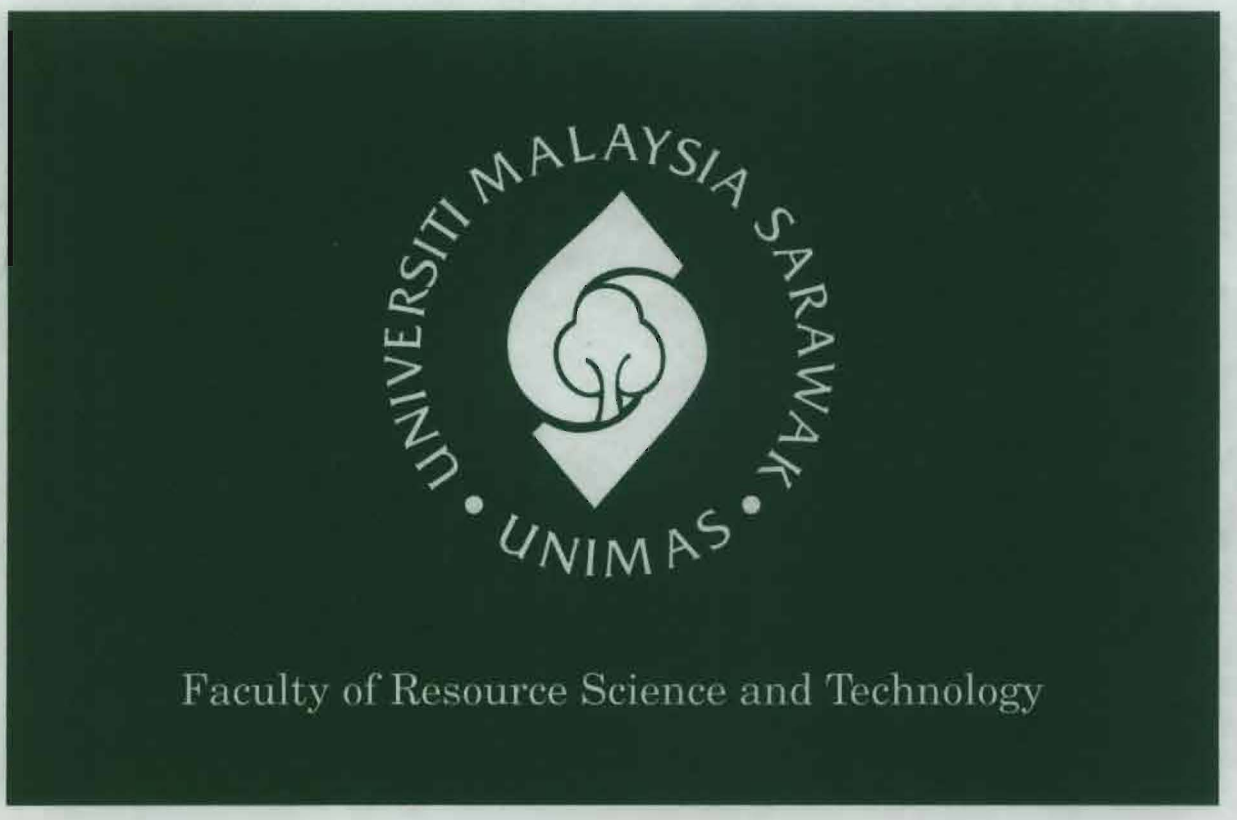

EFFECTS OF CULTURE VESSELS AND ENVIRONMENTS ON MATURATION AND GERMINATION OF SOMATIC EMBRYOS OF SAGO PALM (*METROXYLON SAGU ROTTB.*)

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ABSTRACT

Sago palm (*Metroxylon sagu* Rottb.) is an important economic species and is now grown commercially in Sarawak. Actively multiplying stage 2 somatic embryos of the sago palm supplied by CRAUN (Crop Research and Application Unit) Research Sdn. Bhd. were used to investigate the effects of culture medium and container as well as culture conditions on maturation and germination of sago embryos. The stage 2 somatic embryos were cultured on four different media which were 7CP2 with and without supplement of activated charcoal and DS9 with and without activated charcoal; in each of the three different culture vessels included specimen tube, petri-dish, and conical flask; and placed at three different environments which were low temperature, dark condition, and low light. The somatic embryos were sub-cultured bi-monthly. The results showed that activated charcoal was significantly important to culture embryos of *M. sagu in vitro*, and there was slightly better development for somatic embryos cultured on DS9 media compared to 7CP2 media. Besides that, better results were obtained from embryos cultured in conical flask and petri-dish as compared to specimen tube. However, maturation and germination of sago embryos did not seem to be influenced by the environmental condition.

Key words: Sago palm (*Metroxylon sagu* Rottb.), *in vitro*, somatic embryos, sub-cultured.

ABSTRAK

Pokok sago (Metroxylon sagu Rottb.) merupakan species yang mempunyai kepentingan ekonomi dan kini tumbuh secara kormersial di Sarawak. Somatik embryo pokok sago peringkat 2 yang sedang berkembang dengan aktifnya telah dibekalkan oleh CRAUN (Crop Research and Application Unit) Research Sdn. Bhd., dan digunakan untuk mengkaji kesan daripada beberapa factor fizikal dan keadaan persekitaran yang berbeza terhadap kematangan dan percambahan somatic embryo pokok sago dalam empat media. Somatik embryo peringkat 2 ini telah dikultur dalam empat media yang berlainan di mana terdiri daripada 7CP2 tanpa arang yang diaktifkan, 7CP2 dengan arang yang diaktifkan, DS9 tanpa arang yang diaktifkan, dan DS9 dengan arang yang diaktifkan; dalam tiga bekas kultur yang berlainan, iaitu tabung uji, piring petri, dan kelalang kon; dan diletakkan pada tiga persekitaran yang berlainan iaitu suhu rendah, keadaan gelap, dan cahaya rendah. Somatik embryo ini telah disubkulturkan setiap dua bulan. Keputusan menujukkan bahawa arang yang diaktifkan memainkan peranan yang sangat penting dalam mengkulturkan embryo M. sagu secara in vitro, dan menujukkan sedikit lebih baik percambahan untuk somatik embryo yang dikulturkan dalam DS9 media berbanding dengan 7CP2 media. Selain itu, kelalang kon dan piring petri memperolehi keputusan yang lebih baik berbanding dengan tabung uji. Bagaimanapun persekitaran yang berlainan tidak mempunyai pengaruh yang besar terhadap kematangan dan percambahan somatik embryo pokok sago.

Kata kunci: Pokok sago (Metroxylon sagu Rottb.), in vitro, somatik embryo, disubkulturkan.

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LIST OF ABBREVIATIONS

2,4-D	2,4-Dichlorophenoxylacetic acid
NAA	Napthalene Acetic Acid
BAP	6-benzyl Amino Purine
DS9	25mg/l NAA + 1.0mg/l BAP
7CP2	50mg/l NAA + 2.0mg/l BAP
MS Medium	Murashige and Skoog (1962) medium
PPM™	Plant Preservative Mixture
PVP	Polyviryl Pyrrolidone

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1 INTRODUCTION

" Sago is the gold mine of the 21st century..... our objective is to make sago one of our economic mainstays, capable of earning RM1 billion annually for the state " said by YAB Pehin Sri (Dr.) Haji Abdul Taib Mahmud, the Chief Minister of Sarawak (CRAUN, 1997).

Metroxylon sagu Rottb. is a pinnate-leaved palm. The scientific name is derived from *metra*, meaning pith or *parenchyma*, and *xylon*, meaning *xylem*. A number of different common names for *Metroxylon sagu* exist: in Burmese - *thagu-bin*, in English - *sago palm*, *true sago palm*, in Filipina - *lumbia*, in French - *sagoutier*, in German - *sagopalme*, in Thai - *sa khu*, in Vietnamese - *sa kuu*. In Malaysia, the name *pohon rumbia* is used, and in Sarawak, particularly in Melanau dialect, the local name for *M. sagu* is *balau*. (Flach, 1997)

As explained by McClatchey *et al.* (2006), sago palm reaches 15m (49 ft) in height with bole diameter (without leaf sheaths) of 35-60cm (14-24in) (Plate 1.1). *M. sagu* is occurring in the Southeast Asia, Melanesia, and some islands in Micronesia and Polynesia. A wide range of species can be found in lowland freshwater swamps and in traditional swidden gardens in lowland rain forests. It can grow on a wide variety of soils, including well drained, poor quality sand, peat, clay, or lava. In Malaysia, the only large areas under sago now are in the state of Sarawak on peat which occupies 13.3% of the land area of that state.

Plate1.1 Sago (*Metroxylon sagu*) palm
Source: CRAUN Research Sdn. Bhd.

Fosberg *et al.* (1987) revealed sago palm is an important economic species and is now grown commercially in Malaysia, Indonesia, the Philippines, and New Guinea. The state of Sarawak (East Malaysia) has a long history of sago starch production. Jong (1995) gave an overview of the history of sago starch production for the international market. Sago palm starch has been exported since the early 19th century. Currently, it

ranks fourth among the agricultural export commodities of Sarawak, producing 50, 000 t of air dry starch, worth RM431 millions. The state government of Sarawak is now developing this crop as a plantation crop, utilizing part of the 1.5 million ha of peat swamps. In 1982, the Sarawak state government established a research station specifically for research on peat. The world's first large-scale commercial plantation of 7700 ha of sago was developed by the Sarawak Land Custody and Development Authority (LCDA) near Mukah. In 1993, a second plantation of 1600 ha was established near Oya, in Sarawak.

Flach (1997) mentioned that sago is used as food, for example the boles of sago palm have always been used to obtain starch as a staple food for humans. The growing point and the young leaves around it are generally known as the palm heart or cabbage may be used as vegetable. Ground pith is sometimes used as an animal feed (Fodder), especially for pigs, and when dried, for horses and chickens. Dextrose sugar extract from sago palm starch is a source of fuel, for example it can be processed to yield ethanol. The cortex of the trunk is also used for firing in paper mills. The bark may be used as a domestic fuel after drying. Processing of the pith to yield starch produces a fiber. The leaves also yield a fiber, which may be used for mats. Other usages of sago are in the cultivation of the rice-straw mushroom (*Volvaria volvacea*) which utilizes the waste from sago extraction, the frond rachis is often used for fastening between horizontal posts in walls, and the bark may be used as a flooring material. Sago palm has been planted in buffer zones as a method of rehabilitating degraded lands, for instance the coastal plains of Indonesia where thousands of hectares of land had been abandoned. The waste from pith processing is used as a fertilizer or in other words as soil improver.

Sago starch is a valuable source of carbohydrates widely used in the food industries. Products that had been derived from sago starch are monosodium glutamate, high fructose syrup, glucose, maltose, dextrose, caramel, vinegar, bakers' yeast and products for local food industries. Recent research has shown that product such as maltrodextrin, cyclodextrin, modified sago starch, biodegradable plastic and absorbent, and animal feed can be produced. Sago starch has also been used as a base for medicines and cosmetic.

To date it has been possible to initiate embryogenesis (stage 1) and to multiply the somatic embryos (stage 2) on 2,4-D-free medium but maturation and germination of somatic embryos into plantlets is still extremely poor. Therefore, the research question is what are the effects of types of culture vessels, light intensity and other environmental factors on maturation and germination of somatic embryos of sago palm on four media?

The objective of this study was initiated to investigate the effect of some physical factors and environmental conditions on maturation and germination of somatic embryos on four media. The physical factors include culture vessels, i.e. specimen tubes, Petri-dishes, and conical flasks. Environmental conditions such as dark, low light (300-400 lux) and low temperature (8°C) also play a significant role.

2 LITERATURE REVIEW

2.1 Propagation of *Metroxylon sagu*

Sago palm propagates itself both vegetatively and sexually. *M. sagu* reproduces via vegetative suckers emerging from roots or lower trunks of parent plants and also by stolons. Suckers are then separated from the original trunk, forming new clusters (Schuiling and Jong, 1996). Flach (1997) described *M. sagu* also propagates itself sexually at the end of its life cycle. The inflorescence may contain up to 850 000 fruits. The seed germinates only under wet conditions. A rosette grows from the seed, out of which a new bole and new suckers may be formed.

2.2 *In Vitro* Culture of *Metroxylon sagu*

Krishnapillay and Alang (1986) mentioned sago palm is usually propagated from suckers because vegetative propagation gives a more uniform planting material. At maturity, an undisturbed mother palm will have a clump of 3-10 palms of different ages surrounding it. Since the number of suckers per palm is limited, it is not possible to accelerate the establishment of a pure mono-clonal *M. sagu* planting. In addition, with the increased demand from industry for its products and a 9 to 15 year growth cycle, *M. sagu* will need to be planted on a large scale. The possibility of using tissue culture for vegetative propagation of these palms holds great potential for establishment of this crop on a plantation basis and also in breeding and agronomic trials, and it is the most suitable means for producing the vast amount of planting material required for extensive plantations (Alang *et al.*, 1993 cited by Mashelkar, n.d.).

Plant tissue culture can be referred as the science of growth and development of axenic plant cells, tissues or organs isolated from the mother plant on nutrient medium. The principle of plant *in vitro* regeneration is totipotency which means the ability of a plant cell to develop into whole plant (Trigiano and Gary, 1996).

Clonal propagation of plants *in vitro* provides a means of mass production of selected planting material (Zaliha *et al.*, 2005). This can be through organogenesis or embryogenesis. Somatic embryogenesis can be generally classified into direct embryogenesis and indirect embryogenesis via an intermediate callus phase. These pathways have been used successfully to mass propagate three economically important palms: oil palm, coconut and the date palm. (Krishnapillay and Alang, 1986). Efforts are now underway with respect to mass production of elite clones of *M. sagu*. This is to support the development of the *M. sagu* industry in Malaysia. Currently there is a demand for a least 1.8 million sago palm planting materials by the year 2010 in Sarawak alone and should *M. sagu* be developed as a major commodity, demand for planting material will clearly increase (Zaliha *et al.*, 2005).

The advantages of tissue culture of *M. sagu* are it can create a large number of planting material, and it is possible and easier to select desirable traits directly from the culture setup (*in vitro* selection), thereby decreasing the amount of space required for field trials. The time required is much shortened as there is no need to wait for the whole life cycle of seed development, it allows for the international exchange of sterilized plant materials (eliminating the need for quarantine), and it helps to eliminate plant diseases

through careful stock selection and sterile techniques. In addition, it enables cold storage of large numbers of viable plants in a small space.

A considerable amount of preparatory work has been done on *in vitro* culture of the *M. sagu*. Alang *et al.*, 1993 cited by Mashelkar, n.d. revealed that since 1983, research has been conducted at the Department of Biotechnology, Faculty of Food Science and Biotechnology, Universiti Pertanian Malaysia, Serdang, Selangor, towards clonal propagation of *M. sagu*. Several hundred clonal plantlets were produced over a period of 18-24 months from culture initiation; the protocol for inducing embryogenesis from explants and developing embryoids into plantlets was being improved. Growth of tissue and of embryos *in vitro* has been proved to be possible, but callus formation of *M. sagu* has not yet been achieved (Hisajima *et al.*, 1991).

Henry (1992) revealed in callus culture, only 6% of the explants from embryos and 3% of the explants from leaf and stem tissues produced callus. Induction of callus from roots to become embryogenic had not been successful.

In 1995, research was concentrated on improving the rate of callus initiation from young unopened leaf segments, meristem of *in vitro* germinated seedlings and healthy vigorously growing suckers, but the rate of production was very low (2%). This may be due to the high mortality rate because of injury caused during excision. Conditions for the development of embryoids into plantlets were eratic and difficult to determine. Although complete plantlets were formed, only 42 out of the 100 plantlets hardened in the nursery survived. These too, were slow growing and weak (Julaihi, 1995).

In an *in-vitro* (tissue culture) research, it was reported (Chang, 1997) that Eeuwens basal salt mixture supplemented with 2,4-D, amino acids and biotin resulted in more cultures developing callus or callus-like growth in experiments using meristematic domes of young suckers as explants for callus initiation in *M. sagu*.

The percentage of sago palm cultures that developed callus improved with the addition of amino acids as in the Y3 formulation. Eeuwens basal salt composition was recommended as less browning of the explants was observed. Calli from apical meristematic regions were vigorous and readily became embryogenic. The young embryoids were very sensitive and were often damaged after transfer to subculture. More than 1200 developing embryoids have been isolated for regeneration. Altogether five experiments totaling about 2,500 cultures were done to induce callus formation from leaf segments. The effects of several media supplements were examined. Callus was formed but these were not amenable to further subculture and did not become embryogenic (Chang, 1998).

Chang (1999) stated the experiment was also done to examine the effects of pH on root growth of sago palm plantlets. Sterilised peat medium with pH ranging from 3 – 5 to simulate the natural acidity of peat areas was imposed. Preliminary results indicated pH 3 and 4 were prohibitive not only to root formation but also the shoot development. Generally vegetative growth was poor, root formation inhibited and plantlet regeneration slow. A trial to examine the effect of amino acids on the development of roots on embryoids was done. In terms of root growth (number and branching) development

appeared to be better when amino acids L-Arginine, L-Asparagine and L-Glutamine were added.

Sepuan (2000) disclosed callus formation from meristem tips remained low and was less than 5%. Root development of plantlet *in vivo* remained difficult. Even after roots were formed, plantlet establishment in the soil was poor with very high mortality. This will ultimately be the weak link in developing the protocol for mass propagation of *M. sagu*. Leaf segment explants remained problematic. Although browning could be reduced, the calli obtained were not amenable to subculture and hence could not be induced to become embryogenic.

Zaliha *et al.* (2005) reported a technique for *in vitro* propagation through somatic embryogenesis has been developed and approximately 1,500 clonal *M. sagu* palms were field planted in the first half 2005.

Shoot tips of vigorous offshoots from quality palms are cultured on MS medium, pH 5.5 containing 2.0 g/l activated charcoal, 6% sucrose, 2.2 g/l Gelrite™, 0.1 g/l inositol and a cocktail of auxins and cytokinins, and the explants are cultured at 24°C – 26°C in the dark (Zaliha *et al.*, 2005).

Masni (personal communication, 2006) explained a successful method for *in vitro* propagation of sago palm through somatic embryogenesis has been developed and thousands of clonal palms have recently been planted in field trials on deep peat. Clonal palms from the earliest trial show normal growth and development with trunk initiation occurring within the expected timeframe (4- 6 years after planting). Starch accumulation